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Organically produced coffee exerts protective effects against the micronuclei induction by mutagens in mouse gut and bone marrow

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ABSTRACT

While researchers have extensively evaluated the beneficial effects of coffee consumption in reducing the frequency of certain diseases, studies examining the differences between organic and conventional coffee intake are still needed. Therefore, this paper aims to investigate the functional effects of organic and conventional coffee by examining both its chemical composition and its mutagenic/antimutagenic properties.
Infusions of 10% or 20% (w/v) of organic and conventional coffee were administered by gavage (10 mL/kg b.w., once or twice a day) to male Swiss mice against doxorubicin (DXR) and 1,2-dimethylhydrazine dihydrochloride (DMH)-induced mutagenicity. The levels of chlorogenic acids, caffeine and trigonelline from the coffee infusions and oxidative stress analysis from the liver were measured by HPLC. Gut and bone marrow micronucleus assays were used as mutagenic/antimutagenic endpoints, as well as the crypt measurements and gut apoptosis index. The *in vivo* tests revealed that only organic coffee exerted protective effects, despite oxidative stress analysis and crypt measurements not showing differences among treatments. Intriguingly, the low dose (10% w/v mL/kg) displayed a robust protective effect that showed a significant reduction in bone marrow micronuclei (26.8%), gut micronuclei (11.5%) and apoptosis (27.8%), whereas the higher coffee dose (2 × 20% w/v) only showed a protective effect against bone marrow micronucleus (43.7%). These results highlight that organic coffee could be considered to have beneficial functional effects, although it is still a challenge to define conclusions from analytical data and all the possible interactions from this complex food matrix.

1. Introduction

There are many controversies over the effects of organic coffee, especially in regards to their effects on human health of its chemical contaminants and nutritional composition. Previous studies have demonstrated few and inconsistent differences in the nutrient composition of organically produced foods when compared to foods produced by conventional methods (Bourn & Prescott, 2002; Herencia, García-Galavís, Dorado, & Maqueba, 2011). Despite these controversies, the perception among consumers is that organically produced crops possess higher nutritional quality; this added value results in prices that are minimally 20% higher than crops produced on non-organic farms (Dos Santos, Dos Santos, & Conti, 2009). Currently, Brazil is the major world producer of coffee, and incorporates the three different agricultural methods for coffee cultivation, *i.e.*, organic, traditional and technological procedures (ICO, 2012).

Coffee is the third most widely consumed beverage in the world, after water and tea (Villanueva et al., 2006). It is a complex mixture of bioactive compounds that contain the original coffee constituents, such as caffeine, caffeoyl quinic acids (CQAs) and trigonelline, along with compounds formed during roasting, such as N-methylpyridinium (NMP), nicotinic acid, nicotinamide and melanoidins (Lang, Yagar, Eggers, & Hofmann, 2008). These compounds act as radical scavengers, inducing the expression of antioxidant enzymes, in addition to exhibiting metal chelating activity, observed in different *in vitro* and *in vivo* bioassays (Bakuradze et al., 2010). Evidence is gradually revealing that a high coffee consumption may reduce the risk of some types of

Abbreviations: DXR, doxorubicin; bw, body weight; i.p., Intraperitoneally; DMH, 1,2-dimethylhydrazine dihydrochloride; MN, micronuclei; PS, physiological saline; BMG, body mass gain; SGR, specific growth rate; FCR, feed conversion ratio; OC, organic coffee; CC, conventional coffee; PCE, polychromatic erythrocytes; HE, hematoxylin–eosin; AI, apoptosis index; TBA, thiobarbituric acid; TEP, 1,1,3,3-tetraethoxypropane; MDA, malondialdehyde; DNPH, dinitrophenylhydrazine; MNPCE, micronucleated polychromatic erythrocytes; PCO, protein carbonyl content; HDL, high density lipoprotein; non-HDL, non-high density lipoprotein.

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human cancer, and this risk reduction is mainly associated with its antioxidant activities (Nkondjock, 2009).

Coffee consumption has also been associated with a variety of adverse effects that cannot be ignored. Major health concerns are the addiction to caffeine and potential for withdrawal syndrome, increased central nervous system activity, increased anxiety, insomnia, and potential for lower birth weight in pregnancy (Dorea & Da Costa, 2005). Additionally, carcinogenic compounds, such as polycyclic aromatic hydrocarbons, can also be formed by the incomplete combustion of organic matter during roasting. Fortunately, these carcinogenic compounds have been detected only at insignificant quantities in brewed coffee (Orecchio, Ciotti, & Culotta, 2009). Furthermore, many studies enumerate the potentiating effect that coffee and caffeine have on mutagenesis mediated by both chromosomal aberrations and the shortened repair time of chromosomal damage induced by other mutagenic agents (Nehlig & Debry, 1994). Subsequently, while mutagenic studies on conventional coffee are contradictory, such studies are non-existent for the effects of organic coffee and should be performed.

Among the various techniques used to detect genetic and genotoxic effects, the micronucleus assay is widely applicable for different cell types with potential for detection of both aneugens and clastogens (Kirsch-Volders et al., 2011). Although the micronucleus test is most frequently used to evaluate bone marrow, the gut micronucleus assay considers the gastrointestinal tract and its contact with food. For these assays, the potent mutagens and carcinogens DXR (Dhawan, Kayani, Parry, Parry, & Anderson, 2003) and DMH (Poul, Jarry, Elhkim, & Poul, 2009; Suzuki et al., 2009; Vanhauwaert, Vanparys, & Kirsch-Volders, 2001) are widely used. DXR is a cytotoxic and mutagenic agent that induces micronucleus formation in mammalian system through its clastogenic and aneugenic effects (Dhawan et al., 2003). This micronuclei formation is mediated through the accumulation of reactive oxygen species (Kiyomiya, Matsuo, & Kurebe, 2001), the stabilization of the topoisomerase II–DNA complex, and the enzymatic inhibition of DNA-dependent protein (Guano et al., 1999; Wassermann, 1996). In contrast, the colon carcinogen DMH is considered a potent alkylating and clastogenic compound that induces point mutations, micronuclei formation, sister chromatid exchanges, methyl adducts of DNA bases and apoptosis in the colonic epithelial cells (Newell & Heddle, 2004).

Because of the health implications of coffee drinking, as well as the scarcity of data on the difference between organic and conventional production, our research evaluates the functional effects, chemical composition and mutagenic/antimutagenic aspects of organic *versus* conventional coffee production.

2. Materials and methods

2.1. Coffee samples and coffee infusions

For assays, we used samples of roasted ground *Coffea arabica* L. cv. Mundo Novo from the 2009/2010 crop, which were naturally processed, rated as hard grade (78 points), and medium roasted (55# agtron) in a commercial fluidized bed roaster (i-Roast model no. 40009, USA, Hearthware, 210–220 °C) for 8 min. The organic and conventional samples were provided by the Associação de Pequenos Produtores de Poço Fundo, Minas Gerais, Brazil, and the organic coffee was certified (BCSOKO Garantie Master Certificates nº A-2007-00308/2010-02629). To characterize these coffee powders, an analysis of the coffee powder was performed in triplicate as follows: moisture content was determined by exposure to infrared radiation at 120 °C for 8 min (IAL, 2008); fat was measured using the Bligh and Dyer (1959) method; protein was assessed using the Kjeldahl procedure (conversion factor 6.25); and ash content was determined by incineration at 550 °C in a muffle furnace, and carbohydrates were calculated from the remainder (the difference using the fresh weight-derived) by AOAC (2005). The infusions used in the present study were prepared by adding 5 g or 10 g of coffee powder to 50 mL of water heated at 90 °C and then filtered through a paper filter (pore diameter 14 μm).

2.2. Analysis of chlorogenic acid, caffeine and trigonelline

Coffee infusions and chemical standards (*i.e.*, chlorogenic acids, caffeine and trigonelline) were dissolved in methanol (2.0 mg/mL) (n = 3). The analysis was performed using a SHIMADZU PROMINENCE high-performance chromatograph coupled to a UV–visible (UV/vis) spectrophotometric detector (model SPD-M20A), a SIL-20A power injector and a C-18 VC-ODS RP18 175 column of 25 cm. The mobile phase consisted of H_2O/CH_3COOH (95/5 v/v) (A) and acetonitrile (B) and used the following gradient elution from A/B: 0 min, 95/5; 5 min, 95/5; and 10 min, 87/13; the phase also had a flow rate of 0.7 mL/min. The UV/vis signal detection was programmed as follows: 0–15 min, 272 nm; 15–23 min, 320 nm; and 23–40 min, 272 nm (Alves, Dias, Benassi, & Scholz, 2006).

2.3. Animals, treatment and growth performance

The animals used in this study were handled in accordance with the Ethical Principles for Animal Research adopted by the Brazilian College of Animal Experimentation (COBEA) with a protocol approved by the University's Ethical Committee for Animal Research (protocol nº. 316/2010). Male Swiss mice were obtained from CEMIB (UNICAMP Campinas, SP, Brazil) and were approximately 4–5 weeks of age. Mice were fed *ad libitum* with a commercial pellet diet (Fri-lab Ratos II®) and water. The mice were divided into 10 ten groups of 10 or 20 animals per group (Table 1). The groups received different infusions of coffee or water by oral gavage of 10 mL/kg twice a day for 15 days. Before the end of the experiment (24 h), half of the animals from groups 1 to 7 (n = 10 animals) received Doxorubicin chloridate i.p. (DXR — Rubidox®, Bergamo Laboratory; 30 mg/kg), and the other half (n = 10 animals) received an oral gavage of 1,2-dimethylhydrazine dihydrochloride (DMH, Sigma, St. Louis, MO, USA) (30 mg/kg). Both treatments comprised a single dose with a volume of 10 mL/kg. Groups 8 to 10 (n = 10 animals) received only a physiological solution (PS, NaCl 0.9% w/v). The animals that received DXR treatment (n = 10) were used for the bone marrow MN analysis, and the animals (n = 10) that received DMH treatment were used for the gut MN test, apoptosis analysis and morphometric measurements. At the end of the study, all animals were anesthetized with ketamine and xylazine and then euthanized by exsanguination. During necropsy, the bone marrow

Table 1
Experimental design for the evaluation of the mutagenic/antimutagenic effects of organic/conventional coffee beverages.

Groups (n)	Treatments (first gavage)	Treatments (second gavage)	*In vivo* test	
			Bone marrow assay**	Gut assay**
G1*	Water	Water	DXR	DMH
G2*	OC 10% (w/v)	Water	DXR	DMH
G3*	OC 20% (w/v)	Water	DXR	DMH
G4*	OC 20% (w/v)	OC 20% (w/v)	DXR	DMH
G5*	CC 10% (w/v)	Water	DXR	DMH
G6*	CC 20% (w/v)	Water	DXR	DMH
G7*	CC 20% (w/v)	CC 20% (w/v)	DXR	DMH
G8**	Water	Water	PS	
G9**	OC 20% (w/v)	Water	PS	
G10**	CC 20% (w/v)	Water	PS	

DMH: 1,2-dimethylhydrazine dihydrochloride(30 mg/kg b.w.); DXR: doxorubicin (30 mg/kg b.w.); PS: physiological solution (NaCl 0.9% w/v); OC: organic coffee; CC: conventional coffee; bone marrow assay: MN test; Gut assay: MN test, apoptosis and morphometric measurements.
* n = 20.
** n = 10.

cells, liver and colon were removed from all animals. Blood was centrifuged at 1000 ×*g* for 10 min to obtain serum, and the liver was perfused with PS; these materials were then stored at −18 °C. The colon was removed, opened longitudinally and fixed flat in buffered formalin for 24 h, then placed in 70% (v/v) ethanol. For the growth profile assay, the mice were weighed individually on three days. The body mass gain (BMG), specific growth rate (SGR) and feed conversion ratio (FCR), were calculated for each mouse individually, according to previous reports by Kumar, Akinleye, Makkar, Angulo-Escalante, and Becker (2011).

2.4. In vivo bone marrow MN test, in vivo gut MN test, apoptosis and morphometric measurements

For the mutagenicity and antimutagenicity analysis of different coffee infusions, the bone marrow micronucleus (MN) test was used according to the protocol of Macgregor et al. (1987). Two thousand polychromatic erythrocytes (PCE) were analyzed per mouse in slides blindly scored using a light microscope at 1000× magnification (Venâncio, Silva, Almeida, Brigagão, & Azevedo, 2012). For the gut micronucleus test, morphometric measurements and apoptosis analysis, the colons were excised, flushed with 0.9% NaCl to remove fecal debris, cut open longitudinally and rolled from caecum to anus, as described by Moolenbeek and Ruitenberg (1981). These "Swiss rolls" were fixed in 10% (v/v) neutral formalin, embedded in paraffin and sectioned thorough the roll (5 μm). For the *in vivo* gut micronucleus test, we followed the methods described in the Vanhauwaert et al. (2001), using adjusted coloring conditions to obtain a better contrast of the nucleus against the cellular material. For this purpose, the slides were subjected to hydrolysis by being dipped for 15 min at room temperature in HCl 5 N, rinsed in distilled water three times, immersed in the dark in Schiff's reagent (Merck, Germany) for 90 min and then washed for 5 min in flowing water. After being rinsed in water, the slides were counterstained with fast-green (Vetec Química Fina LTDA, Brazil) (0.5% w/v) for 4 min and rinsed in absolute ethanol. For each animal, 1000 colonic epithelial cells and the total number of crypts analyzed were scored manually using a light microscope at 1000× magnification. For the morphometric and apoptosis analyses, Swiss roll slides were stained with hematoxylin–eosin (HE) and assayed under light microscopy at 400X magnification (Levin et al., 1999). The images were digitalized, and the colonic crypt depth and area were measured (μm) using the UTHSCSA ImageTool program (developed at the University of Texas Health Science Center at San Antonio, Texas and available from the Internet by anonymous FTP from ftp://maxrad6.uthscsa.edu). For the measurements of the crypt depth and crypt area were considered, respectively, the height from the baseline to the crypt surface and the crypt perimeter, following the whole cell row for both of the two measurements. For the crypt measurements and the identification of apoptotic cells, a total of 20 perpendicular well-oriented crypts were examined in each animal, counting the total number of epithelial cells in each one (Chang, Chapkin, & Lupton, 1997). The apoptotic cells were identified as previously described by Risio et al. (1996). The apoptosis index (AI%) was estimated as the percentage of apoptotic cells in relation to the total number of cells counted.

2.5. Biochemical parameters and oxidative stress

To determine the biochemical parameters, peripheral blood samples were centrifuged at 1000 ×*g* for 10 min to obtain serum. The total levels of triglycerides, cholesterol and their fractions were determined using an enzymatic method based on the Trinder reaction (Burtis & Ashwood, 1999). For the analyses of oxidative stress, the liver was removed, homogenized with phosphate buffer, and centrifuged. Liver supernatants were used for the determination of lipid peroxidation and protein carbonyl (Jones, Abdalla, & Freitas, 1995). Samples (40 μL) were mixed with H_3PO_4 (1.22 mol/L, 200 μL), aqueous TBA (0.67% (w/v), 200 μL), and H_2O (Milli-Q, 360 μL). This mixture was heated in a boiling water bath for 60 min. After chilling on ice, alkaline methanol (360 μL MeOH + 40 μL 1 mol/L NaOH) was added to the 200 μL sample, and the samples were centrifuged (2500 ×*g*, 3 min). The neutralized reaction mixture (20 μL) was then chromatographed on a 250 mm × 4.6 mm i.d. VC-ODS RP18 175 column with 50:50 (v/v) 25 mmol/L phosphate buffer and pH 6.5 methanol, using mobile phase with a flow rate 0.8 mL/min. The fluorimetric detection was performed at λexc532 nm and λemm553 nm, using a model RF-10AXL detector. The peak of the MDA-TBA adduct was calibrated with a TEP standard solution processed in exactly the same way as the samples (Brown & Kelly, 1996). The concentration of MDA was calculated using a standard curve for MDA, and all of the results were expressed as μmol MDA/mg protein. The protein carbonyl content (PCO) was measured by forming labeled protein hydrazone derivatives using DNPH that were then quantified spectrophotometrically (Levine et al., 1990). The carbonyl content was determined from the absorbance at 370 nm using a molar absorption coefficient of 21,000 M^{-1} cm^{-1} and expressed as nmol/mg protein (Reznick & Packer, 1994). The protein content was quantified by Bradford's Assay (Bradford, 1976).

2.6. Statistical analysis

Differences in the frequency of micronucleated and apoptotic cells among different experimental groups (*i.e.*, control, different sampling of coffees, different doses) were compared using the Mann–Whitney *U*-test (two-tailed). Evaluations of mean body weight, oxidative stress, morphometric measurements and biochemical parameters were performed using a one-way analysis of variance (ANOVA), followed by Tukey's test. The results were considered statistically significant if P values were 0.05 or less. The percentage of reduction in the micronucleus of bone marrow and gut as well as the apoptotic cells, was calculated according to Azevedo et al. (2003).

3. Results

The results of the centesimal composition measurements did not reveal any difference between organic and the conventional management, so the concentrations were as follows: carbohydrate (67.16 ± 0.88 g/100 g), ash (4.44 ± 0.05 g/100 g), lipids (12.60 ± 0.65 g/100 g), proteins (12.22 ± 0.23 g/100 g) and moisture (3.59 ± 0.16 g/100 g).

For the measurements of the bioactive compounds, the retention times for the standards of chlorogenic acids, caffeine and trigonelline were 22.599, 23.290 and 2.907 min, respectively. The linearity of the method was 1 to 22 μg/mL for chlorogenic acid, 5 to 25 μg/mL for caffeine and 1 to 12 μg/mL for trigonelline. The limits of detection/quantification obtained were 0.15/0.52 μg/g for chlorogenic acid, 0.45/1.51 μg/g for caffeine and 0.63/2.11 μg/g for trigonelline, respectively. Organic and conventional management did not disclose any differences in the composition of these compounds, which varied as follows: chlorogenic acids (1.36 ± 0.04–1.99 ± 0.39 mg/g), caffeine (18.17 ± 0.29–21.41 ± 0.04 mg/g) and trigonelline (7.31 ± 0.27–7.67 ± 0.21 mg/g).

Additionally, we found no significant differences in the parameters of nutritional growth performance. The variation of the nutritional profile for all experimental groups is presented below: initial body mass (20.07 ± 2.50–25.04 ± 1.35 g), final body mass (28.30 ± 3.36–32.00 ± 2.58 g), BMG (%) 22.24 ± 8.10–51.09 ± 15.19, SGR (% per day) 1.12 ± 0.66–2.76 ± 0.35, FCR 10.08 ± 1.99–27.66 ± 17.12 and liver mass 1.41 ± 0.23–1.71 ± 0.25 g.

To measure the oxidative stress, we found the linearity of this method for the MDA assay to be 0.24–4.8 μg/mL, and the limits of detection/quantification were 0.15/0.50 μg/g. These results showed a significant increase in MDA and protein carbonyl levels between

the group that received DXR (G1) and the negative controls (G4), as follows: MDA (G1-DXR = 58.8 ± 14.3 nmol/mg protein and G4 = 35.6 ± 8.4 nmol/mg protein) and PCO (G1 = 2.20 ± 0.80 nmol/mg protein and G4 = 1.09 ± 0.61 nmol/mg protein). When we compared the DMH (G1-DMH) treatment group to the negative control group (G4), we only found an increase in PCO (G1-DMH = 1.90 ± 0.10 nmol/mg protein and G4 = 1.09 ± 0.61 nmol/mg protein). Furthermore, groups subjected to infusions of coffee that also received DMH and DXR (G2–G7) treatment showed no difference in MDA and PCO levels (data not shown). The biochemical parameters did not indicate any differences among all experimental groups, and these data varied as follows: triglycerides 117.26 ± 35.99–145.38 ± 22.61 mg/dL, total cholesterol 105.63 ± 12.82–139.00 ± 21.21 mg/dL, cholesterol HDL 52.53 ± 12.38–81.60 ± 12.59 mg/dL and non-HDL cholesterol 42.05 ± 10.11–64.98 ± 17.67 mg/dL.

In the bone marrow (Fig. 1A) and gut assay tests (Fig. 1B and C), we found significant increases in the micronuclei frequencies and of total apoptotic cells of the positive controls groups (G1 — DXR or DMH) in comparison to the negative control group (G4) (Tables 2 and 3). In all of the groups treated with DXR, we also observed a decline in the PCE/NCE ratio when compared with negative controls. Fig. 2 highlights the profile of protective effect that is observed only in the organic management condition (G2, G3, G4), with damage reduction shown in relation to the respective controls (G1 — DXR or DMH). Thus, we found reductions in MNPCE (26.8–43.7%) for all three coffee concentrations, reductions in the apoptosis index (27.8 and 18.2, respectively) for both the 10% and 20% OC infusion concentrations, and reductions in the micronucleus gut test (11.5%) for the 10% OC infusion. In the crypt morphometric measurements, the differences were not significant for experimental and control groups, and these data varied to depth 182.86 ± 17.44–201.24 ± 29.25 μm/crypt and the area 13.13 ± 1.92–15.23 ± 3.25 mm^2/crypt.

4. Discussion

For this investigation, both conventional and organic coffee brews were harvested during the 2009/2010 season and were produced under the same conditions, such as the geographic region, variability of external factors (*e.g.*, sun light, temperature and rain), storage, transportation and processing. The type of agriculture management had no consequences in terms of the concentration of specific compounds, such as chlorogenic acids, caffeine and trigonelline. This is contradictory to our previous findings (Carvalho et al., 2011), where higher levels of these same components were observed when organic management was employed. Differences in the chemical and nutritional composition between management types are discussed in the literature and can be found even in the same crop that is cultivated in different years (Carvalho et al., 2011, Herencia et al., 2011).

In the *in vivo* experiments, we applied coffee infusions in amounts equivalent to the average consumption of the population (10%, 20% and 2 × 20% w/v), closely mimicking the level of human intake. To maintain the effectiveness of the coffee components and to keep the coffee solution fresh during the experiment, the coffee infusions were prepared daily, and the roasted coffee grains were ground twice per week. The monitored variables of body-weight, body-weight gain, food intake and biochemical serum characteristics are useful indicators of toxicity, and showed that the nutritional profile of the animals was not affected by the consumption of any type of coffee infusion.

Examination of the mutagenic/antimutagenic effects showed that 24 h after the oral administration of DXR and DMH, the number of nuclear aberrations in the bone marrow (Table 2) and gut epithelial cells (Table 3) was considerably increased when compared to the untreated mice, indicating a sensitivity to the test systems (Çelik, Mazmanci, Çamlica, Aşkin, & Çömelekoğlu, 2005; Vanhauwaert et al., 2001). Additionally, treatment groups did not display any mutagenic effects when compared to the negative and positive controls.

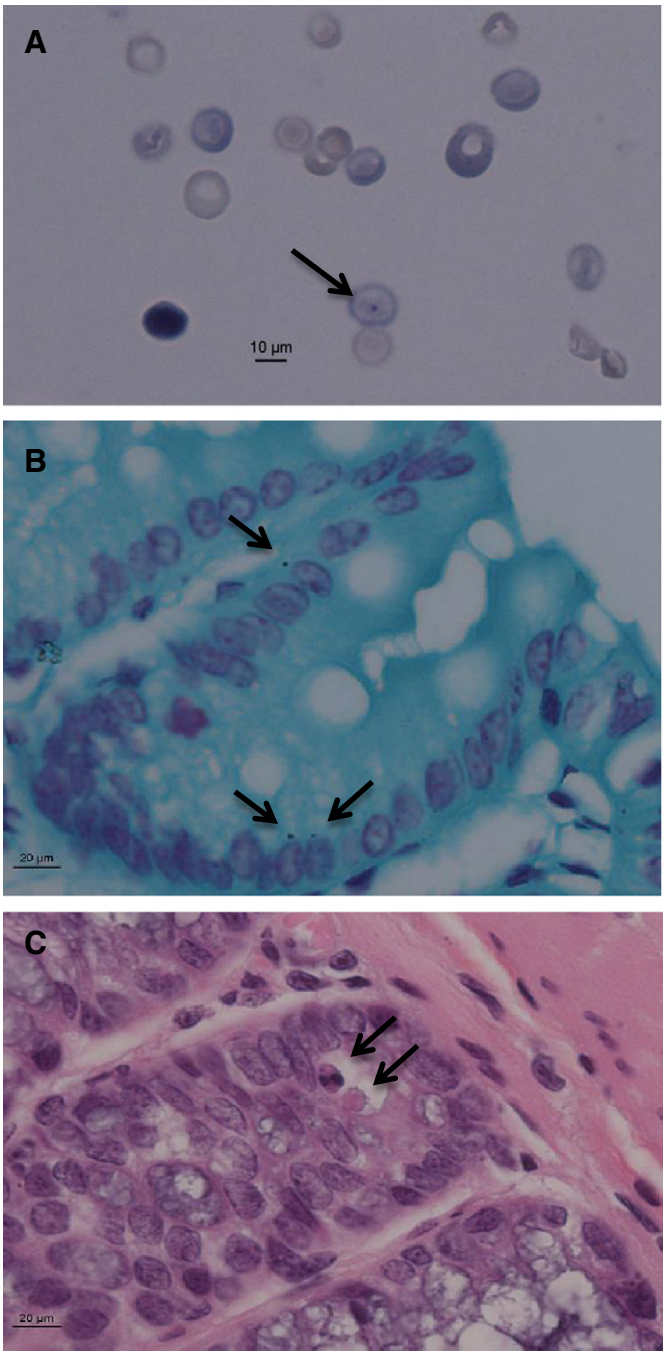

Fig. 1. *Description*: Profile of images of damage assessed in the bone marrow and gut. Views of different types of damage identified in polychromatic erythrocyte and colonic epithelium 24 h after 30 mg/kg DMH and DXR. (A) Micronucleated PCE (arrow, 1000× objective), (B) micronucleated colonocytes (arrows, 1000×). (C) Colonocytes in apoptosis (arrows, 1000×).

In contrast, only the organic coffee infusions protected against DXR and DMH-induced cytotoxicity, as observed by the reduction in the bone marrow (micronucleus) and colon parameters (micronucleus and apoptosis), respectively (Fig. 2).

In this study, we included the analysis of gut epithelial cells because they are the first to come in contact with food compounds and are highly suitable for the MN evaluation due to elevated cell turnover (Vanhauwaert et al., 2001). For short-term mutagenicity/genotoxicity assays, the bone marrow micronucleus test has been widely used to identify noxious chemicals; however, the detection of potential genotoxicants for other target-tissues cannot be assured

Table 2
The effects of organic and conventional coffee on the frequencies of micronucleated polychromatic erythrocytes (MNPCE) in the bone marrow of male Swiss mice.

Groups/Treatment	N	Cells	PCE/NCE	MNPCE	%MN
G1/DXR	10	20,000	1.34	323	1.62
G2/OC 10% (w/v) + DXR	10	20,000	1.29	252*	1.33
G3/OC 20% (w/v) + DXR	10	20,000	1.40	253*	1.26
G4/OC 2 × 20% (w/v) + DXR	10	20,000	1.46	209*	1.05
G5/CC 10% (w/v) + DXR	10	20,000	1.28	307	1.54
G6/CC 20% (w/v) + DXR	10	20,000	1.56	328	1.64
G7/CC 2 × 20% (w/v) + DXR	10	20,000	1.38	315	1.58
G8/PS	10	20,000	1.90	62	0.31
G9/OC 20% (w/v) + PS	06	12,000	1.76	44	0.37
G10/CC 20% (w/v) + PS	06	12,000	1.75	47	0.39

DXR: doxorubicin (30 mg/kg b.w.); PS: physiological solution (NaCl, 0.9% w/v); OC: organic coffee; CC: conventional coffee; PCE/NCE = polychromatic erythrocytes/normochromatic erythrocytes ratio; MN = micronuclei; N = 2000 analyzed cells/animal.
Conclusions: G2 < G1, G3 < G1 and G4 < G1; *P < 0.05 (Mann–Whitney *U*-test) compared with positive control (G1).

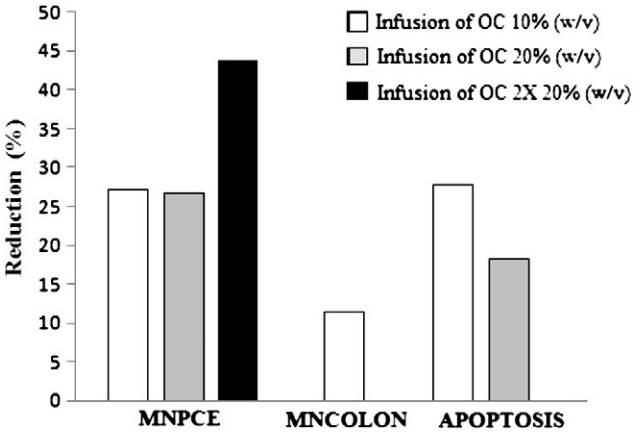

Fig. 2. *Description*: This figure shows the main results about the protective effects from organic coffee brew obtained from *in vivo* experiment. Micronuclei (MN) and apoptosis reduction (%) in groups treated with infusions of 10%, 20%, or 2 × 20% of either conventional or organic coffees when compared to positive control.

(Kirsch-Volders et al., 2011; Vanhauwaert et al., 2001). The micronucleus test on gut epithelial cells is able to detect both clastogenic and aneugenic effects in chemical compound products that are not detected by the bone marrow micronucleus test (Vanhauwaert et al., 2001). Despite the disadvantage of the improper positioning of histological sections, which might inadvertently omit micronuclei formation (Ohyama et al., 2002), the gut epithelial cell micronucleus test showed a greater homogeneity of MN data within each group (CV bone marrow = 46.13%; CV gut = 37.25%) when compared to the bone marrow assay. These methods were complementary and showed different responses. In the bone marrow test, all concentrations of organic coffee exerted a protective effect; however, in the gut cell micronucleus test, only the lower dose (10% w/v,) displayed this protective effect.

Apoptosis is an important event in the eradication of cells suffering from DNA insult due to mutagenic/genotoxic chemicals or radiation exposure (Nowsheen & Yang, 2012). Therefore, an increase or facilitation of apoptosis during chemical insult consequently increases the elimination of mutated cells that might otherwise progress to malignancy after a long period of latency (Bartek & Lukas, 2007). Interestingly, we observed protective effects in the apoptosis analysis for the two lower concentrations of organic coffee infusions (10% and 20% w/v). In this case, the lowest concentration (10% w/v) presented the greatest inhibition of apoptosis and consequently had the highest reduction of deleterious effect (28%). This variety of results may be due to the specific actions of DXR, DMH on various cell types, which may elicit different responses from bioactive compounds in these systems. Furthermore, Bjelakovic, Nikolova, Gluud, Simonetti, and Gluud (2007) using studies with antioxidants, reinforce the conclusion that the lower consumption of some compounds and their synergism with others in the diet may have a better protective function than at higher levels of ingestion.

Because we used a coffee beverage instead of specific coffee components, the ability of organic coffee to protect against the clastogenic and aneugenic effects of DMH and DXR may also be mediated by the impact of its complex mixture, containing thousands of different chemical entities, on putative antimutagenic or anticarcinogenic effects (Nehlig & Debry, 1994). These effects could be attributed to the chemoprotective compounds found in coffee infusions, such as alkaloids (caffeine and trigonelline), phenolic compounds (chlorogenic acids, catechins and anthocyanins), and diterpenes (cafestol and kahweol) (Minamisawa, Yoshida, & Takai, 2004; Oestreich-Janzen, 2010; Vignoli, Bassoli, & Benassi, 2011). Kahweol and cafestol have been previously reported to protect against well-known carcinogens (Kim, Jung, & Jeong, 2004) and inhibit the mutagenicity/tumorigenicity of several carcinogens through specific modifications in xenobiotic metabolism, such as cytochrome P450 and sulfotransferase in rat liver (Huber et al., 2002; Huber et al., 2008). Furthermore, the DNA methylation caused by DMH in the gut epithelial cells is also inhibited by caffeic acid, as demonstrated previously in cultured MCF-7 and MAD-MB 231 human cancer cells (Vucic, Brown, & Lam, 2008).

Although the production of OH• free radicals and lipid peroxidation by DXR contributes to the major induction of MN mechanisms (Venkatesh, Bellary, Ganesh, Rao, & Manjeshwar, 2007), we did not observe the antioxidant effects of these infusions in our study. One possibility is that the protective effects of all doses against DXR in the micronucleus test occurred through other metabolic pathways

Table 3
Results of *in vivo* gut epithelial cells micronuclei test and apoptotic cells.

Groups	Treatments	Frequency of micronucleated gut epithelial cells					Frequency of apoptotic cells		
		Total crypts	Total cells	Cell/crypt	Total micronucleated cells	% MN	Total apoptotic cells	Total cells	AI%
G1	DMH	212	9000	42.45	213	2.37	61	7188	0.85
G2	OC 10%(w/v) + DMH	245	10,000	40.82	200	2.00*	46*	7205	0.64
G3	OC 20%(w/v) + DMH	240	10,000	41.67	248	2.48	47*	7438	0.63
G4	OC 2 × 20%(w/v) + DMH	234	10,000	42.74	237	2.37	52	7198	0.72
G5	CC 10%(w/v) + DMH	247	10,000	40.49	230	2.30	53	7084	0.75
G6	CC 20%(w/v) + DMH	241	10,000	41.49	239	2.39	68	7285	0.93
G7	CC 2 × 20%(w/v) + DMH	235	10,000	42.55	240	2.40	60	7258	0.82
G8	PS	227	9000	39.65	90	0.90	7	7660	0.09
G9	OC 20%(w/v) + PS	208	9000	43.27	76	0.84	2	6742	0.03
G10	CC 20%(w/v) + PS	212	9000	42.45	79	0.87	7	6672	0.10

DMH: 1,2-dimethylhydrazine dihydrochloride(30 mg/kg b.w.); PS: physiological solution (NaCl, 0.9% w/v); OC: organic coffee; CC: conventional coffee; MN: micronucleated cells; AI: Apoptosis Index.
Conclusions: for gut micronuclei test, G2 < G1; for apoptosis analysis, G2 < G1 and G3 < G1. *P < 0.05 (Mann–Whitney *U*-test) compared with positive control (G1).

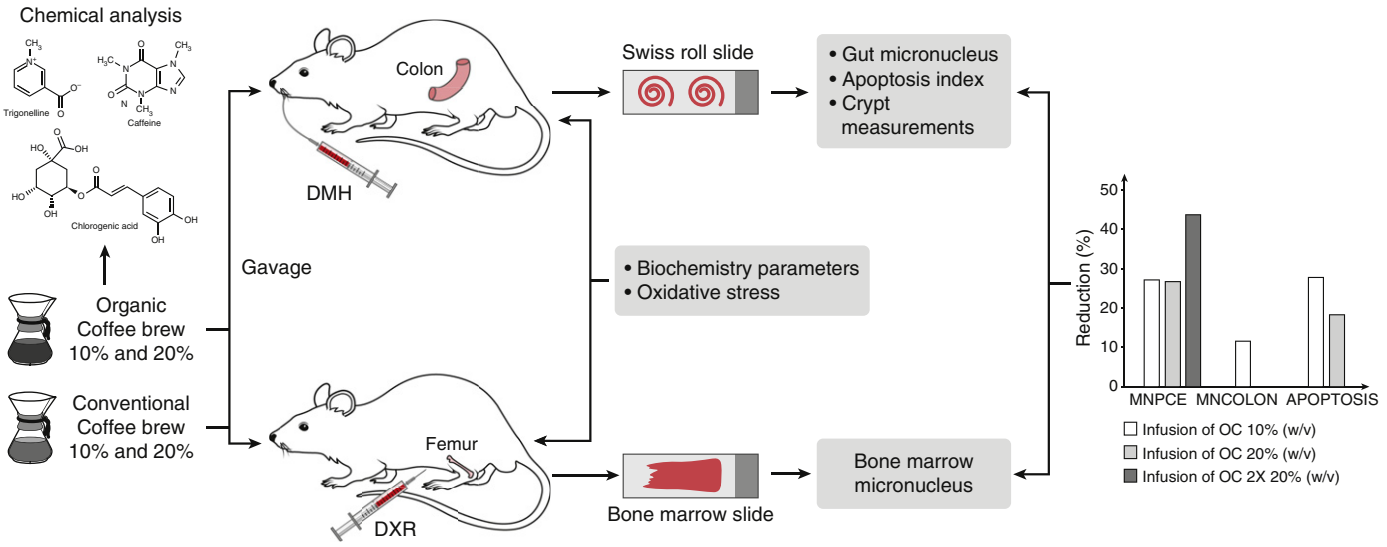

Fig. 3. *Description*: This figure represents the graphical abstract of the study with organic and conventional coffee brew. Effects of organically and conventionally produced coffee against DXR and DMH induction *in vivo*.

of this drug as enzymatic inhibition of DNA-dependent protein. Thus, the differences in our results from bone marrow and gut tissues may be due to many factors, such as the balance among compounds and their tissue bioavailability. The balance between the chemical composition of the green beans is influenced by management and roasting (*e.g.*, trigonelline *vs.* polycyclic aromatic hydrocarbons) (Esquivel & Jiménez, 2012; Herencia et al., 2011; Orecchio et al., 2009). In conjunction, the bioavailability of these elements may be modified as a consequence of their interactions with each other in the infusions and their interactions with the macronutrients of the food in the gastrointestinal tract (Duarte & Farah, 2011; Dupas, Baglieri, Ordonaud, Tomé, & Maillard, 2006). Another possible hypothesis for the protective effect is that organic food increases the capacity of living organisms towards resilience (Huber, Rembialkowska, Średnicka, Bügel, & Van de Vijver, 2011). However, the roles of these bioactive compounds are not well defined in the literature.

5. Conclusions

The overall number of studies comparing the nutritional value of organic and conventional foods is growing and there is also increasing interest in investigating the health effects of organic food consumption (Huber et al., 2011). Taken together, our results show that the lower dose (10 mL/kg) was protective in all systems (*i.e.*, bone marrow and gut micronuclei, apoptosis), whereas the higher dose (2×20 mL/kg) was only protective against bone marrow micronucleus (graphical abstract is shown in Fig. 3). These results highlight that the studied compounds elicited different effects on both different drugs and systems. According to Esquivel and Jiménez (2012), it is difficult to draw conclusions from analytical data about the health effects of mainly organic foods; however, we here provide evidence that organic coffee could have considerable functional effects on general health.

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